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(54) Title: DNA SEQUENCES ENCODING THE HUMAN A1, A2a and A2b ADENOSINE RECEPTORS

(57) Abstract

The present invention relates to DNA sequences encoding the human A1, A2a and A2b adenosine receptors. In addition, the present invention relates to the use of these DNA sequences in the production of human A1, A2a and A2b adenosine receptors using recombinant DNA technology.

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DNA Sequences Encoding the Human A1, A2a and A2b Adenosine Receptors

Field of the Invention

The present invention relates to DNA sequences encoding the human A1, A2a and A2b adenosine receptors. In addition, the present invention relates to the use of these DNA sequences in the production of the human A1, A2a and A2b adenosine receptors using recombinant DNA technology.

Background of the Invention

Adenosine influences cardiovascular function (by slowing heart rate and decreasing blood pressure) and also influences nervous system function (through sedative and anti-epileptic effects). In addition, adenosine can induce bronchoconstriction. Adenosine binds specifically to at least three receptors, A1 and A2a and A2b. Adenosine receptors have been shown to couple to a number of second messenger systems. Additional adenosine receptor subtypes may exist. As adenosine receptor agonists and antagonists may have commercial value as anti-hypertensive agents, hypnotics, anti-psychotics and bronchodilators, the ability to produce adenosine receptors by recombinant DNA technology is advantageous.

The present inventors have isolated three related cDNA fragments encoding the human A1, A2a and A2b adenosine receptors from human hippocampal cDNA by using either the polymerase chain reaction and unique degenerate oligonucleotides to generate specific probes or by using specific consensus oligonucleotide probes for cDNA library screening. Full-length cDNA clones for each of the three receptors were isolated from a human hippocampal cDNA library. The receptor sequences were identified as the human A1, A2a and A2b adenosine receptors by expression in mammalian cells and both measurement of the affinity of the encoded receptors for various adenosine analogues and

the effect of receptor activation on cAMP synthesis. The receptors have homology to cDNA's encoding the dog A1 and A2a adenosine receptors (MAENHAUT, C., VAN SANDE, J., LIBERT, F., ADRAMOWIC, M., PARMENTIER, M., VANDERHAEGEN, J., DUMONT, D., VASSART, G. AND SCHIFFMANN, S. (1990); LIBERT, F., SCHUFFMANN, S.M., LEFORT, A., PARMENTIER, M., GERARD, C., DUMONT, J.E., VANDERHAEGHEN J.J., VASSART, G. (1991)) and the rat A2b adenosine receptor (STEHLE, J.H., RIVKEES, S.A., LEE, J.J., WEAVER, D.R., DEEDS, J.D. AND REPPERT, S.M. (1992)). These hippocampal cDNA sequences represent novel human receptors which may be of clinical and commercial importance.

Summary of the Invention

Accordingly, in a first aspect the present invention consists in a DNA molecule encoding the human A1 adenosine receptor, the DNA molecule having a sequence substantially as shown in Figure 1 or a functionally equivalent sequence.

In a second aspect the present invention consists in a DNA molecule encoding the human A2a receptor subtype, the DNA molecule having a sequence substantially as shown in Figure 2 or a functionally equivalent sequence.

In a third aspect the present invention consists in a DNA molecule encoding the human A2b adenosine receptor subtype, the DNA molecule having a sequence substantially as shown in Figure 3 or a functionally equivalent sequence.

As used herein the term "functionally equivalent sequence" is intended to cover variations in the DNA sequence which, due to degeneracy of the DNA code, do not result in the sequence encoding a different polypeptide. Further, this term is intended to cover alterations in the DNA code which lead to changes in the encoded polypeptide, but in which such changes do not affect the biological activity of the polypeptide.

As used herein the term "DNA molecule" is intended to

cover both genomic DNA and cDNA.

In a fourth aspect the present invention consists in a method of producing the human A1 adenosine receptor comprising culturing a cell transformed with the DNA molecule of the first aspect of the present invention under conditions which allow expression of the DNA sequence such that the human A1 adenosine receptor is expressed on the cell surface and optionally recovering the human A1 adenosine receptor.

In a fifth aspect the present invention consists of a method of producing a human A2a adenosine receptor comprising culturing a cell transformed with the DNA molecule of the second aspect of the present invention under conditions which allow expression of the DNA sequence such that the human A2 adenosine receptor is expressed on the cell surface and optionally recovering the human A2a adenosine receptor.

In a sixth aspect the present invention consists of a method of producing a human A2b adenosine receptor comprising culturing a cell transformed with the DNA molecule of the third aspect of the present invention under conditions which allow expression of the DNA sequence such that the human A2 adenosine receptor is expressed on the cell surface and optionally recovering the human A2b adenosine receptor.

In further aspects the present invention consists of a method of screening a molecule for adenosine agonist or antagonist activity, comprising contacting the molecule with the human A1, A2a or A2b adenosine receptors produced by the method of the fourth, fifth or sixth aspect of the present invention.

In yet a further aspect the present invention consists in oligonucleotides 305, 377 and 376 as hereinafter described.

The DNA molecules of the present invention represent

novel human receptors. These receptors may be of interest both clinically and commercially as they are expressed in many regions of the body and as adenosine affects a wide number of systems.

The isolated full-length DNA clones containing the complete coding region for these receptors can be used to establish mammalian cell lines producing the receptors for use in agonist and antagonist screening. The receptor DNA sequence can be used for additional homology screening to identify novel members of this receptor family.

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following examples and figures in which:-

Figure 1 shows the nucleotide and amino acid sequence of the human A1 adenosine receptor cDNA.

Figure 2 shows the nucleotide and amino acid sequence of the human A2a adenosine receptor cDNA.

Figure 3 shows the nucleotide and amino acid sequence of the human A2b adenosine receptor cDNA.

Figure 4A shows saturation isotherms of the total (unfilled triangle), specific (filled circle) and non-specific (unfilled square) binding of the A1 adenosine receptor antagonist DPCPX (8-cyclopentyl-1,3 dipropylxanthine) to mammalian CHO.K1 cells expressing the human A1 adenosine receptor.

Figure 4B shows competition binding curves showing the displacement of CGS-21680 2-p-(2-Carboxyethyl)phenethylamino-5'-N-ethylcarboxyamido adenosine hydrochloride) by different adenosine agonists and antagonists (NECA = 5'-N-ethylcarboxamido adenosine; CA=2-chloroadenosine; CPA=N^6-cyclopentyladenosine; XAC=xanthine amine congener; T=8-(p-sulphophenyl)-theophylline) in mammalian HEK 293 cells expressing the human A2a adenosine receptor.

Figure 5 shows the effects of the different adenosine receptor subtypes, A1, A2a and A2b upon cyclic AMP production. A1 adenosine receptor activation leads to inhibition of forskolin stimulated cAMP levels. Activation of both the A2a and A2b adenosine receptors (by CGS-21680 and NECA, respectively) leads to stimulation of cAMP levels.

METHODS

Oligonucleotide Design and Synthesis

Unique degenerate oligonucleotides corresponding to the transmembrane II (TM II) and IV (TM IV) regions of G protein-coupled receptors and containing either a 5' EcoRI restriction enzyme site (TM II oligonucleotide 377) or a 3' Hind III restriction enzyme site (TM IV oligonucleotides 305 and 376) were synthesized on an Applied Biosystems automated DNA synthesiser. The sequences of the oligonucleotides are as follows:-

305 5' - CCCAATAAGCTTAGICCIATGGCGAAAGACAGGACCCA-3'
A A G G C
A A

376 5' - GAGTCCGAAGCTTAGTGGGCAAGAGATGGCGAAIGAIAGIACCA-3'
G TA C A G
T A

377 5' - CAGAACGAATTCAATGTTTTTATGTGGTCTTTGTCITCIACTGA-3'
C G G G G G C
A

The DNA sequences included inosine (I) residues. Crude oligonucleotides were then used in the polymerase chain reaction.

PCR Amplification

Sequences homologous to the G protein-coupled

receptor oligonucleotides were amplified from human cDNA using PCR and the Hybaid thermocycler. DNA was prepared from a human neuroblastoma (Clontech) cDNA library in lambda gt10 and from a hippocampal (Stratagene) cDNA library in lambda ZapII. DNA was prepared by phenol and chloroform extraction of approximately 10^8 library phage and ethanol precipitation to recover the DNA. DNA from the cDNA libraries (1-5μg) was incubated with 200μM of each dNTP, 0.5μM oligonucleotide, 0.5 units Tth enzyme (Toyobo) in 50mM KCl, 50mM Tris-HCl pH9.0, 1.5mM $MgCl_2$ (1 x PCR buffer) in a 50μL reaction volume. Samples were layered with 50μL light mineral oil (Sigma). Reactions were denatured for 5 minutes at 95°C. The PCR conditions were as follows: Denaturation for 2 minutes at 92°C, annealing for 2 minutes at 55°C, and extension for 2 minutes at 92°C, 2 minutes at 50°C, and 2 minutes at 70°C, repeated five times; then 2 minutes at 95°C, 2 minutes at 45°C, and 2 minutes at 70°C, repeated thirty times.

Subcloning and Sequencing of Amplified DNA Fragments

Amplified DNA (20μl) was removed and analysed by gel electropheresis in 1% agarose and 3% NuSieve (SeaKem). Amplification products 260bp-330bp in length were excised from the gel and purified with Geneclean. DNA fragments were then digested with Hind III for one hour at 37°C and EcoRI for one hour at 37°C, the DNA again purified with Geneclean and eluted into 10μl H_2O. Digested DNA fragments were then subcloned into M13mp19 and sequenced by the Sanger dideoxy chain-termination method using the Pharmacia or the Promega DNA sequencing kit. Sequencing reactions were analysed on a 6% acrylamide, 7M urea gel, dried onto Whatman 3M paper, and exposed to X-ray film for sixteen hours (Kodak X-OMAT AR5) at room temperature overnight.

Sequence Analysis of Novel DNA Sequences

Sequence analysis of the DNA fragments generated from the PCR amplification identified two DNA fragments that had sequences common to other known G protein-coupled receptors. PCR amplification of neuroblastoma cDNA with the degenerate oligonucleotides 377 and 305 produced a cDNA fragment which was designated 3.1. PCR amplification of human hippocampal cDNA with the degenerate oligonucleotides 377 and 376 produced a cDNA fragment with a sequence that was 76% homologous at the nucleotide level to sequence 3.1 and was designated 3.2 The DNA sequences were searched on the GenBank and EMBL databases for comparison to known sequences and were confirmed to be novel sequences with a high level of homology to dog adenosine A1 and A2 receptors.

Isolation of Full-Length cDNA Clones

Full-length cDNA clones encoding the A1 receptor as well as receptor sequences corresponding to 3.1 and 3.2 were isolated from a human hippocampal cDNA library (Stratagene).

A1 adenosine receptor cDNA isolation

Specific consensus oligonucleotides corresponding to the second extracellular loop (679) and to the third intracellular loop (678) were synthesised on an Applied Biosystems automated DNA synthesiser. The sequences of the oligonucleotides are as follows:-

678 5 - CCCGTAGTACTTCTGCGGGTCGCCAGAGGAGGCGACACCTTCTTGCC-3'

679 5'-GAGGCGCAGCGGGCCTGGGCGGCCAACGGCAGCGGCGGCGAGCCCGTG-3'

Approximately 5 x 10^5 plaques were plated on C600 HflA bacterial cells. Plaques were lifted on to Hybond-N+nylon filters (0.45μM, 137mm, Amersham). DNA was denatured on the filters with a 3 minute incubation on 0.5 M NaOH, 1.5M NaCl and neutralised with a 5 minute incubation in 0.5M Tris pH72, 1mM EDTA and 1.5M NaCl. DNA

was fixed to the filters with a 15 minute exposure to 0.4M NaOH. Filters were then rinsed in 2 x SSC (3M NaCl, 0.3M sodium citrate) and allowed to dry before a 30 minute prehybridisation in 40% formamide, 5 x SSC, 5 x Denhardt's, 50mM $NaPO_4$, 0.5% sodium dodecyl sulphate (SDS), 0.1mg/ml salmon sperm DNA at room temperature. Oligonucleotides 678 and 679 were pooled and 50 pmoles total were radiolabelled using γ^{32}P-ATP and the DNA 5' end-labelling system (Promega). The filters were hybridised with this radiolabelled probe overnight at 42°C, after which time they were washed once briefly in 2 x SSC at room temperature then twice for 10 minutes each wash in 2 x SSC, 0.1SDS at room temperature with a final wash in 0.1 x SSC, 0.1%SDS for 15 minutes at 50°C. The filters were then exposed to Kodak X-OMAT AR5 film overnight at -70°C. Over twenty pure phage isolates which hybridised to the radiolabelled 678 and 679 oligonucleotides were obtained. Several of these different cDNAs were sequenced. The sequence of one such cDNA (together with the deduced amino acid sequence) which encodes the human A1 adenosine receptor is shown in Figure 1.

A2a and A2b adenosine receptor cDNA isolation

Approximately 1 x 10^6 plaques were plated on C600HflA bacterial cells. Plaques were lifted onto Hybond-N nylon filters (0.45μM, 137mm, Amersham). DNA was denatured on the filters with a 3 minute incubation on 0.5M NaOH, 1.5M NaCl and neutralised with a 7 minute incubation in 0.5M Tris pH 7.2, 1mM EDTA and 1.5M NaCl. Filters were rinsed in 2 x SSC (20 x SSC is 3M NaCl, 0.3M sodium citrate) and DNA fixed to the filters with a 5 minute exposure to ultraviolet light (312nm). Filters were prehybridied in 5 x SSPE (5 x SSPE=0.5M NaCl, 0.05M NaH_2PO_4, 0.0005M EDTA, pH 7.7), 5 x Denhardt's (0.1% (w/v) bovine serum albumin, 0.1% (w/v) Ficoll, 0.1% (w/v)

polyvinylpyrollidone), 0.5% sodium dodecyl sulphate (SDS), 0.2mg/ml salmon sperm DNA at 65°C for 17 hours. The filters were hybridised with a radiolabelled probe corresponding to the PCR amplified DNA fragment encoding the 300 bp of 3.1 (labelled with (α-^{32}P)-dCTP using the random primers DNA labelling system (Bethesda Research Laboratories)). Following hybridisation of the radiolabelled probe for 20 hours at 65°C, filters were washed with 2 x SSPE, 0.1% SDS at room temperature for 10 minutes, then with 1 x SSPE, 0.1% SDS at room temperature for 10 minutes and exposed to Kodak X-OMAT AR5 film for seven days at -70°C. Two pure phage isolates were hybridised to the radiolabelled 3.1 DNA fragment were obtained. The two DNA inserts were excised form the phage vector using EcoRI digestion and subcloned into M13mp19 for sequencing. Sequence analysis indicated that one cDNA insert of approximately 2.6 kilobases encoded the full-length clone for the 3.2 receptor. The sequence of the cDNA (together with the putative amino acid sequence) insert encoding the 3.1 receptor (the human A2a adenosine receptor) is shown in Figure 2 (together with the deduced amino acid sequence of the human A2a adenosine receptor) whilst the sequence of the cDNA insert encoding the 3.2 receptor (the human A2b adenosine receptor) is shown in Figure 3 (together with the deduced amino acid sequence).

Expression of the cloned A1, A2a and A2b adenosine receptors in mammalian cells

Each cloned full-length cDNA was subcloned into a mammalian cell expression vector (pcDNAlneo for A2a and A2b and pRc/CMV for A1 (Invitrogen)) in such a way as to direct expression of the encoded receptor portion.

Mammalian cell lines (Chinese Hamster Ovary - CHO K1 or Human Embryonic Kidney - HEK 293) were independently transfected with the recombinant expression vectors and cell lines established which had stably integrated the

cloned receptor DNA. The stably transfected cell lines were examined for their ability to bind a range of adenosine analogues as shown in Figure 4. Furthermore, the effect on cyclic AMP (cAMP) levels of receptor activation by adenosine agonists was examined as shown in Figure 5.

These studies demonstrate that cDNA clone 3.1 encodes an adenosine A2a receptor, cDNA clone 3.2 encodes an adenosine A2b receptor and that the A1 cDNA encodes an adenosine A1 receptor. Generation of significant amounts of purified receptor protein, made possible by this invention, can be used as a tool to facilitate the design and chemical synthesis of highly specific agonists and antagonists for each receptor subtype. Knowledge of the primary sequence differences between the related receptor subtypes as determined by this invention provides crucial information for the design of receptor subtype specific agonists and antagonists.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:-

1. A DNA molecule encoding the human A1 adenosine receptor, the DNA molecule having a sequence substantially as shown in Figure 1 or a functionally equivalent sequence.

2. A DNA molecule encoding the human A2a receptor subtype, the DNA molecule having a sequence substantially as shown in Figure 2 or a functionally equivalent sequence.

3. A DNA molecule encoding the human A2b adenosine receptor subtype, the DNA molecule having a sequence substantially as shown in Figure 3 or a functionally equivalent sequence.

4. A method of producing the human A1 adenosine receptor comprising culturing a cell transformed with the DNA molecule as claimed in Claim 1 under conditions which allow expression of the DNA sequence such that the human A1 adenosine receptor is expressed on the cell surface and optionally recovering the human A1 adenosine receptor.

5. A method of producing a human A2a adenosine receptor comprising culturing a cell transformed with the DNA molecule as claimed in Claim 2 under conditions which allow expression of the DNA sequence such that the human A2a adenosine receptor is expressed on the cell surface and optionally recovering the human A2a adenosine receptor.

6. A method of producing a human A2b adenosine receptor comprising culturing a cell transformed with the DNA molecule as claimed in Claim 3 under conditions which allow expression of the DNA sequence such that the human A2b adenosine receptor is expressed on the cell surface and optionally recovering the human A2b adenosine receptor.

7. A method of screening a molecule for adenosine agonist or antagonist activity, comprising contacting the molecule with the human A1, A2a and A2b adenosine receptors produced by the method as claimed in any one of Claims 3 to 6.

Sequence Range: 1 to 1290

```
          10          20          30          40
           *           *           *           *
CGC AGG ATG GTG CTT GCC TCG TGC CCC TTG GTG CCC GTC TGC TGA TGT

 50          60          70          80          90
  *           *           *           *           *
GCC CAG CCT GTG CCC GCC ATG CCG CCC TCC ATC TCA GCT TTC CAG GCC
                        Met Pro Pro Ser Ile Ser Ala Phe Gln Ala>

  100         110         120         130         140
    *           *           *           *           *
GCC TAC ATC GGC ATC GAG GTG CTC ATC GCC CTG GTC TCT GTG CCC GGG
Ala Tyr Ile Gly Ile Glu Val Leu Ile Ala Leu Val Ser Val Pro Gly>

    150         160         170         180         190
      *           *           *           *           *
AAC GTG CTG GTG ATC TGG GCG GTG AAG GTG AAC CAG GCG CTG CGG GAT
Asn Val Leu Val Ile Trp Ala Val Lys Val Asn Gln Ala Leu Arg Asp>

        200         210         220         230         240
          *           *           *           *           *
GCC ACC TTC TGC TTC ATC GTC TCG CTG GCG GTG GCT GAT GTG GCC GTG
Ala Thr Phe Cys Phe Ile Val Ser Leu Ala Val Ala Asp Val Ala Val>

          250         260         270         280
            *           *           *           *
GGT GCC CTG GTC ATC CCC CTC GCC ATC CTC ATC AAC ATT GGG CCA CAG
Gly Ala Leu Val Ile Pro Leu Ala Ile Leu Ile Asn Ile Gly Pro Gln>

290         300         310         320         330
  *           *           *           *           *
ACC TAC TTC CAC ACC TGC CTC ATG GTT GCC TGT CCG GTC CTC ATC CTC
Thr Tyr Phe His Thr Cys Leu Met Val Ala Cys Pro Val Leu Ile Leu>

  340         350         360         370         380
    *           *           *           *           *
ACC CAG AGC TCC ATC CTG GCC CTG CTG GCA ATT GCT GTG GAC CGC TAC
Thr Gln Ser Ser Ile Leu Ala Leu Leu Ala Ile Ala Val Asp Arg Tyr>

    390         400         410         420         430
      *           *           *           *           *
CTC CGG GTC AAG ATC CCT CTC CGG TAC AAG ATG GTG GTG ACC CCC CGG
Leu Arg Val Lys Ile Pro Leu Arg Tyr Lys Met Val Val Thr Pro Arg>

        440         450         460         470         480
          *           *           *           *           *
AGG GCG GCG GTG GCC ATA GCC GGC TGC TGG ATC CTC TCC TTC GTG GTG
Arg Ala Ala Val Ala Ile Ala Gly Cys Trp Ile Leu Ser Phe Val Val>
```

FIG.1

```
          490         500         510         520
           *           *           *           *
GGA CTG ACC CCT ATG TTT GGC TGG AAC AAT CTG AGT GCG GTG GAG CGG
Gly Leu Thr Pro Met Phe Gly Trp Asn Asn Leu Ser Ala Val Glu Arg>

530         540         550         560         570
 *           *           *           *           *
GCC TGG GCA GCC AAC GGC AGC ATG GGG GAG CCC GTG ATC AAG TGC GAG
Ala Trp Ala Ala Asn Gly Ser Met Gly Glu Pro Val Ile Lys Cys Glu>

  580         590         600         610         620
   *           *           *           *           *
TTC GAG AAG GTC ATC AGC ATG GAG TAC ATG GTC TAC TTC AAC TTC TTT
Phe Glu Lys Val Ile Ser Met Glu Tyr Met Val Tyr Phe Asn Phe Phe>

    630         640         650         660         670
     *           *           *           *           *
GTG TGG GTG CTG CCC CCG CTT CTC CTC ATG GTC CTC ATC TAC CTG GAG
Val Trp Val Leu Pro Pro Leu Leu Leu Met Val Leu Ile Tyr Leu Glu>

       680         690         700         710         720
        *           *           *           *           *
GTC TTC TAC CTA ATC CGC AAG CAG CTC AAC AAG AAG GTG TCG GCC TCC
Val Phe Tyr Leu Ile Arg Lys Gln Leu Asn Lys Lys Val Ser Ala Ser>

          730         740         750         760
           *           *           *           *
TCC GGC GAC CCG CAG AAG TAC TAT GGG AAG GAG CTG AAG ATC GCC AAG
Ser Gly Asp Pro Gln Lys Tyr Tyr Gly Lys Glu Leu Lys Ile Ala Lys>

770         780         790         800         810
 *           *           *           *           *
TCG CTG GCC CTC ATC CTC TTC CTC TTT GCC CTC AGC TGG CTG CCT TTG
Ser Leu Ala Leu Ile Leu Phe Leu Phe Ala Leu Ser Trp Leu Pro Leu>

  820         830         840         850         860
   *           *           *           *           *
CAC ATC CTC AAC TGC ATC ACC CTC TTC TGC CCG TCC TGC CAC AAG CCC
His Ile Leu Asn Cys Ile Thr Leu Phe Cys Pro Ser Cys His Lys Pro>

    870         880         890         900         910
     *           *           *           *           *
AGC ATC CTT ACC TAC ATT GCC ATC TTC CTC ACG CAC GGC AAC TCG GCC
Ser Ile Leu Thr Tyr Ile Ala Ile Phe Leu Thr His Gly Asn Ser Ala>
```

FIG.1 (cont'd.)

```
       920         930         940         950         960
        *           *           *           *           *
ATG AAC CCC ATT GTC TAT GCC TTC CGC ATC CAG AAG TTC CGC GTC ACC
Met Asn Pro Ile Val Tyr Ala Phe Arg Ile Gln Lys Phe Arg Val Thr>

          970         980         990        1000
           *           *           *           *
TTC CTT AAG ATT TGG AAT GAC CAT TTC CGC TGC CAG CCT GCA CCT CCC
Phe Leu Lys Ile Trp Asn Asp His Phe Arg Cys Gln Pro Ala Pro Pro>

1010        1020        1030        1040        1050
  *           *           *           *           *
ATT GAC GAG GAT CTC CCA GAA GAG AGG CCT GAT GAC TAG ACC CCG CCT
Ile Asp Glu Asp Leu Pro Glu Glu Arg Pro Asp Asp ***>

 1060        1070        1080        1090        1100
   *           *           *           *           *
TCC GCT CCC ACC AGC CCA CAT CCA GTG GGG TCT CAG TCC AGT CCT CAC

   1110        1120        1130        1140        1150
     *           *           *           *           *
ATG CCC GCT GTC CCA GGG GTC TCC CTG AGC CTG CCC CAG CTG GGC TGT

      1160        1170        1180        1190        1200
        *           *           *           *           *
TGG CTG GGG GCA TGG GGG AGG CTC TGA AGA GAT ACC CAC AGA GTG TGG

         1210        1220        1230        1240
           *           *           *           *
TCC CTC CAC TAG GAG TTA ACT ACC CTA CAC CTC TGG GCC CTG CAG GAG

1250        1260        1270        1280        1290
  *           *           *           *           *
GCC TGG GAG GGA AGG GTC CTA CGG AGG GAC CAG GTG TCT AGA
```

FIG.1 (cont'd.)

Sequence Range: 1 to 2575

```
          10         20         30         40
          *          *          *          *
CAA TTT TCA GCT GTT CTT TGC TCA ATA ATA ACT TTT TTA TCA CCA AGA

50         60         70         80         90
*          *          *          *          *
TAT CTC TCT AAG TTT TTG ACA TAT TCC TCA TTT GTT TTG ATA AAA GTT

100        110        120        130        140
 *          *          *          *          *
TTC TTA TTT TCT TAG AAA AAT AAG TTA CTA AAA GTC ATA TAT CAT TGT

150        160        170        180        190
 *          *          *          *          *
ATA TCT TCA AAA TAT TGC TTA AAA CTA GGA CTT GTA TTT AAA TGT TTT

200        210        220        230        240
 *          *          *          *          *
TTC TTC TTA AAG ACA ATT TGC AGG TGC CCT CAG GAA CCC TGA AGC TGG

          250        260        270        280
          *          *          *          *
GCT GAG CCA TGA TGC TGC TGC CAG AAC CCC TGC AGA GGG CCT GGT TTC

290        300        310        320        330
 *          *          *          *          *
AGG AGA CTC AGA GTC CTC TGT GAA AAA GCC CTT GGA GAG CGC CCC AGC

340        350        360        370        380
 *          *          *          *          *
AGG GCT GCA CTT GGC TCC TGT GAG GAA GGG GCT CAG GGG TCT GGG CCC

390        400        410        420        430
 *          *          *          *          *
CTC CGC CTG GGC CGG GCT GGG AGC CAG GCG GGC GGC TGG GCT GCA GCA

440        450        460        470        480
 *          *          *          *          *
AAT GGA CCG TGA GCT GGC CCA GCC CGC GTC CGT GCT GAG CCT GCC TGT

          490        500        510        520        530
          *          *          *          *          *
CGT CTG TGG CC ATG CCC ATC ATG GGC TCC TCG GTG TAC ATC ACG GTG GAG
               Met Pro Ile Met Gly Ser Ser Val Tyr Ile Thr Val Glu>

          540        550        560        570
          *          *          *          *
CTG GCC ATT GCT GTG CTG GCC ATC CTG GGC AAT GTG CTG GTG TGC TGG
Leu Ala Ile Ala Val Leu Ala Ile Leu Gly Asn Val Leu Val Cys Trp>
```

FIG.2

```
580          590          600          610          620
 *            *            *            *            *
GCC GTG TGG CTC AAC AGC AAC CTG CAG AAC GTC ACC AAC TAC TTT GTG
Ala Val Trp Leu Asn Ser Asn Leu Gln Asn Val Thr Asn Tyr Phe Val>

  630          640          650          660          670
   *            *            *            *            *
GTG TCA CTG GCG GCG GCC GAC ATC GCA GTG GGT GTG CTC GCC ATC CCC
Val Ser Leu Ala Ala Ala Asp Ile Ala Val Gly Val Leu Ala Ile Pro>

    680          690          700          710          720
     *            *            *            *            *
TTT GCC ATC ACC ATC AGC ACC GGG TTC TGC GCT GCC TGC CAC GGC TGC
Phe Ala Ile Thr Ile Ser Thr Gly Phe Cys Ala Ala Cys His Gly Cys>

      730          740          750          760          770
       *            *            *            *            *
CTC TTC ATT GCC TGC TTC GTC CTG GTC CTC ACG CAG AGC TCC ATC TTC
Leu Phe Ile Ala Cys Phe Val Leu Val Leu Thr Gln Ser Ser Ile Phe>

         780          790          800          810
          *            *            *            *
AGT CTC CTG GCC ATC GCC ATT GAC CGC TAC ATT GCC ATC CGC ATC CCG
Ser Leu Leu Ala Ile Ala Ile Asp Arg Tyr Ile Ala Ile Arg Ile Pro>

820          830          840          850          860
 *            *            *            *            *
CTC CGG TAC AAT GGC TTG GTG ACC GGC ACG AGG GCT AAG GGC ATC ATT
Leu Arg Tyr Asn Gly Leu Val Thr Gly Thr Arg Ala Lys Gly Ile Ile>

  870          880          890          900          910
   *            *            *            *            *
GCC ATC TGC TGG GTG CTG TCG TTT GCC ATC GGC CTG ACT CCC ATG CTA
Ala Ile Cys Trp Val Leu Ser Phe Ala Ile Gly Leu Thr Pro Met Leu>

    920          930          940          950          960
     *            *            *            *            *
GGT TGG AAC AAC TGC GGT CAG CCA AAG GAG GGC AAG AAC CAC TCC CAG
Gly Trp Asn Asn Cys Gly Gln Pro Lys Glu Gly Lys Asn His Ser Gln>

       970          980          990         1000         1010
        *            *            *            *            *
GGC TGC GGG GAG GGC CAA GTG GCC TGT CTC TTT GAG GAT GTG GTC CCC
Gly Cys Gly Glu Gly Gln Val Ala Cys Leu Phe Glu Asp Val Val Pro>

         1020         1030         1040         1050
          *            *            *            *
ATG AAC TAC ATG GTG TAC TTC AAC TTC TTT GCC TGT GTG CTG GTG CCC
Met Asn Tyr Met Val Tyr Phe Asn Phe Phe Ala Cys Val Leu Val Pro>

1060         1070         1080         1090         1100
 *            *            *            *            *
CTG CTG CTC ATG CTG GGT GTC TAT TTG CGG ATC TTC CTG GCG GCG CGA
Leu Leu Leu Met Leu Gly Val Tyr Leu Arg Ile Phe Leu Ala Ala Arg>

  1110         1120         1130         1140         1150
   *            *            *            *            *
CGA CAG CTG AAG CAG ATG GAG AGC CAG CCT CTG CCG GGG GAG CGG GCA
Arg Gln Leu Lys Gln Met Glu Ser Gln Pro Leu Pro Gly Glu Arg Ala>
```

FIG.2 (cont'd.)

```
      1160        1170        1180        1190        1200
       *           *           *           *           *
CGG TCC ACA CTG CAG AAG GAG GTC CAT GCT GCC AAG TCA CTG GCC ATC
Arg Ser Thr Leu Gln Lys Glu Val His Ala Ala Lys Ser Leu Ala Ile>

      1210        1220        1230        1240        1250
       *           *           *           *           *
ATT GTT GGG CTC TTT GCC CTC TGC TGG CTG CCC CTA CAC ATC ATC AAC
Ile Val Gly Leu Phe Ala Leu Cys Trp Leu Pro Leu His Ile Ile Asn>

            1260        1270        1280        1290
             *           *           *           *
TGC TTC ACT TTC TTC TGC CCC GAC TGC AGC CAC GCC CCT CTC TGG CTC
Cys Phe Thr Phe Phe Cys Pro Asp Cys Ser His Ala Pro Leu Trp Leu>

1300        1310        1320        1330        1340
 *           *           *           *           *
ATG TAC CTG GCC ATC GTC CTC TCC CAC ACC AAT TCG GTT GTG AAT CCC
Met Tyr Leu Ala Ile Val Leu Ser His Thr Asn Ser Val Val Asn Pro>

      1350        1360        1370        1380        1390
       *           *           *           *           *
TTC ATC TAC GCC TAC CGT ATC CGC GAG TTC CGC CAG ACC TTC CGC AAG
Phe Ile Tyr Ala Tyr Arg Ile Arg Glu Phe Arg Gln Thr Phe Arg Lys>

      1400        1410        1420        1430        1440
       *           *           *           *           *
ATC ATT CGC AGC CAC GTC CTG AGG CAG CAA GAA CCT TTC AAG GCA GCT
Ile Ile Arg Ser His Val Leu Arg Gln Gln Glu Pro Phe Lys Ala Ala>

            1450        1460        1470        1480        1490
             *           *           *           *           *
GGC ACC AGT GCC CGG GTC TTG GCA GCT CAT GGC AGT GTC GGA GAG CAG
Gly Thr Ser Ala Arg Val Leu Ala Ala His Gly Ser Val Gly Glu Gln>

            1500        1510        1520        1530
             *           *           *           *
GTC AGC CTC CGT CTC AAC GGC CAC CCG CCA GAG GTG TGG GCC AAC GGC
Val Ser Leu Arg Leu Asn Gly His Pro Pro Glu Val Trp Ala Asn Gly>

1540        1550        1560        1570        1580
 *           *           *           *           *
AGT GCT CCC CAC CCT GAG CGG AGG CCC AAT GGC TAC GCC CTG GGG CTG
Ser Ala Pro His Pro Glu Arg Arg Pro Asn Gly Tyr Ala Leu Gly Leu>

      1590        1600        1610        1620        1630
       *           *           *           *           *
GTG AGT GGA GGG AGT GCC CAA GAG TCC CAG GGG AAC ACG GGC CTC CCA
Val Ser Gly Gly Ser Ala Gln Glu Ser Gln Gly Asn Thr Gly Leu Pro>

      1640        1650        1660        1670        1680
       *           *           *           *           *
GAC GTG GAG CTC CTT AGC CAT GAG CTC AAG AGA GTG TGC CCA GAG CCC
Asp Val Glu Leu Leu Ser His Glu Leu Lys Arg Val Cys Pro Glu Pro>

            1690        1700        1710        1720        1730
             *           *           *           *           *
CCT GGC CTA GAT GAC CCC CTG GCC CAG GAT GGA GCA GGA GTG TCC TGA
Pro Gly Leu Asp Asp Pro Leu Ala Gln Asp Gly Ala Gly Val Ser ***>

            1740        1750        1760        1770
             *           *           *           *
TGA TTC ATG GAG TTT GCC CCT TCC TAA G GGA AGG AGA TCT TTA TCT TTC
*** Phe Met Glu Phe Ala Pro Ser ***>
```

FIG.2 (cont'd.)

```
1780        1790        1800        1810        1820
 *           *           *           *           *
TGG TTG GCT TGA CCA GTC ACG TTG GGA GAA GAG AGA GAG TGC CAG GAG

1830        1840        1850        1860        1870
 *           *           *           *           *
ACC CTG AGG GCA GCC GGT TCC TAC TTT GGA CTG AGA GAA GGG AGC CCC

1880        1890        1900        1910        1920
 *           *           *           *           *
AGG CTG GAG CAG CAT GAG GCC CAG CAA GAA GGG CTT GGG TTC TGA GGA

1930        1940        1950        1960        1970
 *           *           *           *           *
AGC AGA TGT TTC ATG CTG TGA GGC CTT GCA CCA GGT GGG GGC CAC AGC

1980        1990        2000        2010
 *           *           *           *
ACC AGC AGC ATC TTT GCT GGG CAG GGC CCA GCC CTC CAC TGC AGA AGC

2020        2030        2040        2050        2060
 *           *           *           *           *
ATC TGG AAG CAC CAC CTT GTC TCC ACA GAG CAG CTT GGG CAC AGC AGA

2070        2080        2090        2100        2110
 *           *           *           *           *
CTG GCC TGG CCC TGA GAC TGG GGA GTG GCT CCA ACA GCC TCC TGC CAC

2120        2130        2140        2150        2160
 *           *           *           *           *
CCA CAC ACC ACT CTC CCT AGA CTC TCC TAG GGT TCA GGA GCT GCT GGG

2170        2180        2190        2200        2210
 *           *           *           *           *
CCC AGA GGT GAC ATT TGA CTT TTT TTC CAG GAA AAA TGT AAG TGT GAG

2220        2230        2240        2250
 *           *           *           *
GAA ACC CTT TTT ATT TTA TTA CCT TTC ACT CTC TGG CTG CTG GGT CTG

2260        2270        2280        2290        2300
 *           *           *           *           *
CCG TCG GTC CTG CTG CTA ACC TGG CAC CAG AGC CTC TGC CCG GGG AGC

2310        2320        2330        2340        2350
 *           *           *           *           *
CTC AGG CAG TCC TCT CCT GCT GTC ACA GCT GCC ATC CAC TTC TCA GTC

2360        2370        2380        2390        2400
 *           *           *           *           *
CCA GGG CCA TCT CTT GGA GTG ACA AAG CTG GGA TCA AGG ACA GGG AGT

2410        2420        2430        2440        2450
 *           *           *           *           *
TGT AAC AGA GCA GTG CCA GAG CAT GGG CCC AGG TCC CAG GGG AGA GGT

2460        2470        2480        2490
 *           *           *           *
TGG GGC TGG CAG GCC ACT GGC ATG TGC TGA GTA GCG CAG AGC TAC CCA

2500        2510        2520        2530        2540
 *           *           *           *           *
GTG AGA GGC CTT GTC TAA CTG CCT TTC CTT CTA AAG GGA ATG TTT TTT

2550        2560        2570
 *           *           *
TCT GAG ATA AAA TAA AAA CGA GCC ACA G
```

FIG.2 (cont'd.)

Sequence Range: 1 to 1687

```
          10          20          30          40
           *           *           *           *
TC AGC CCC GAG GCT CAG AAG CGG CAG GCG GAG GCG CGG TCC GGG CGC

          60          70          80          90
           *           *           *           *
TAT GGC CAT GCC CGG CGG GTC TCA CGC GGC TGC CCC TCG CCC GGC GCG

 100         110         120         130         140
  *           *           *           *           *
CCT TCG GTA GGG GGC GCC CGG GGC CCA GCT GGC CCG GCC ATG CTG CTG
                                                 Met Leu Leu>

    150         160         170         180         190
     *           *           *           *           *
GAG ACA CAG GAC GCG CTG TAC GTG GCG CTG GAG CTG GTC ATC GCC GCG
Glu Thr Gln Asp Ala Leu Tyr Val Ala Leu Glu Leu Val Ile Ala Ala>

       200         210         220         230         240
        *           *           *           *           *
CTT TCG GTG GCG GGC AAC GTG CTG GTG TGC GCC GCG GTG GGC ACG GCG
Leu Ser Val Ala Gly Asn Val Leu Val Cys Ala Ala Val Gly Thr Ala>

          250         260         270         280
           *           *           *           *
AAC ACT CTG CAG ACG CCC ACC AAC TAC TTC CTG GTG TCC CTG GCT GCG
Asn Thr Leu Gln Thr Pro Thr Asn Tyr Phe Leu Val Ser Leu Ala Ala>

290         300         310         320         330
 *           *           *           *           *
GCC GAC GTG GCC GTG GGG CTC TTC GCC ATC CCC TTT GCC ATC ACC ATC
Ala Asp Val Ala Val Gly Leu Phe Ala Ile Pro Phe Ala Ile Thr Ile>

  340         350         360         370         380
   *           *           *           *           *
AGC CTG GGC TTC TGC ACT GAC TTC TAC GGC TGC CTC TTC CTC GCC TGC
Ser Leu Gly Phe Cys Thr Asp Phe Tyr Gly Cys Leu Phe Leu Ala Cys>

    390         400         410         420         430
     *           *           *           *           *
TTC GTG CTG GTG CTC ACG CAG AGC TCC ATC TTC AGC CTT CTG GCC GTG
Phe Val Leu Val Leu Thr Gln Ser Ser Ile Phe Ser Leu Leu Ala Val>

       440         450         460         470         480
        *           *           *           *           *
GCA GTC GAC AGA TAC CTG GCC ATC TGT GTC CCG CTC AGG TAT AAA AGT
Ala Val Asp Arg Tyr Leu Ala Ile Cys Val Pro Leu Arg Tyr Lys Ser>

          490         500         510         520
           *           *           *           *
TTG GTC ACG GGG ACC CGA GCA AGA GGG GTC ATT GCT GTC CTC TGG GTC
Leu Val Thr Gly Thr Arg Ala Arg Gly Val Ile Ala Val Leu Trp Val>
```

FIG.3

```
530         540         550         560         570
 *           *           *           *           *
CTT GCC TTT GGC ATC GGA TTG ACT CCA TTC CTG GGG TGG AAC AGT AAA
Leu Ala Phe Gly Ile Gly Leu Thr Pro Phe Leu Gly Trp Asn Ser Lys>

  580         590         600         610         620
   *           *           *           *           *
GAC AGT GCC ACC AAC AAC TGC ACA GAA CCC TGG GAT GGA ACC ACG AAT
Asp Ser Ala Thr Asn Asn Cys Thr Glu Pro Trp Asp Gly Thr Thr Asn>

    630         640         650         660         670
     *           *           *           *           *

GAA AGC TGC TGC CTT GTG AAG TGT CTC TTT GAG AAT GTG GTC CCC ATG
Glu Ser Cys Cys Leu Val Lys Cys Leu Phe Glu Asn Val Val Pro Met>

      680         690         700         710         720
       *           *           *           *           *
AGC TAC ATG GTA TAT TTC AAT TTC TTT GGG TGT GTT CTG CCC CCA CTG
Ser Tyr Met Val Tyr Phe Asn Phe Phe Gly Cys Val Leu Pro Pro Leu>

          730         740         750         760
           *           *           *           *
CTT ATA ATG CTG GTG ATC TAC ATT AAG ATC TTC CTG GTG GCC TGC AGG
Leu Ile Met Leu Val Ile Tyr Ile Lys Ile Phe Leu Val Ala Cys Arg>

770         780         790         800         810
 *           *           *           *           *
CAG CTT CAG CGC ACT GAG CTG ATG GAC CAC TCG AGG ACC ACC CTC CAG
Gln Leu Gln Arg Thr Glu Leu Met Asp His Ser Arg Thr Thr Leu Gln>

  820         830         840         850         860
   *           *           *           *           *
CGG GAG ATC CAT GCA GCC AAG TCA CTG GCC ATG ATT GTG GGG ATT TTT
Arg Glu Ile His Ala Ala Lys Ser Leu Ala Met Ile Val Gly Ile Phe>

    870         880         890         900         910
     *           *           *           *           *
GCC CTG TGC TGG TTA CCT GTG CAT GCT GTT AAC TGT GTC ACT CTT TTC
Ala Leu Cys Trp Leu Pro Val His Ala Val Asn Cys Val Thr Leu Phe>

      920         930         940         950         960
       *           *           *           *           *
CAG CCA GCT CAG GGT AAA AAT AAG CCC AAG TGG GCA ATG AAT ATG GCC
Gln Pro Ala Gln Gly Lys Asn Lys Pro Lys Trp Ala Met Asn Met Ala>

          970         980         990         1000
           *           *           *           *
ATT CTT CTG TCA CAT GCC AAT TCA GTT GTC AAT CCC ATT GTC TAT GCT
Ile Leu Leu Ser His Ala Asn Ser Val Val Asn Pro Ile Val Tyr Ala>

1010        1020        1030        1040        1050
 *           *           *           *           *
TAC CGG AAC CGA GAC TTC CGC TAC ACT TTT CAC AAA ATT ATC TCC AGG
Tyr Arg Asn Arg Asp Phe Arg Tyr Thr Phe His Lys Ile Ile Ser Arg>

  1060        1070        1080        1090        1100
   *           *           *           *           *
TAT CTT CTC TGC CAA GCA GAT GTC AAG AGT GGG AAT GGT CAG GCT GGG
Tyr Leu Leu Cys Gln Ala Asp Val Lys Ser Gly Asn Gly Gln Ala Gly>
```

FIG.3 (cont'd.)

```
      1110        1120        1130        1140        1150
       *           *           *           *           *
GTA CAG CCT GCT CTC GGT GTG GGC CTA TGA TCT AGG CTC TCG CCT CTT
Val Gln Pro Ala Leu Gly Val Gly Leu ***>

      1160        1170        1180        1190        1200
       *           *           *           *           *
CCA GGA GAA GAT ACA AAT CCA CAA GAA ACA AAG AGG ACA CGG CTG GTT

           1210        1220        1230        1240
            *           *           *           *
TTC ATT GTG AAA GAT AGC TAC ACC TCA CAA GGA AAT GGA CTG CCT CTC

1250        1260        1270        1280        1290
 *           *           *           *           *
TTG AGC ACT TCC CTG GAG CTA CCA CGT ATC TAG CTA ATA TGT ATG TGT

 1300        1310        1320        1330        1340
  *           *           *           *           *

CAG TAG TAG CAC CAA GGA TTG ACA AAT ATA TTT ATG ATC TAT TCA GCT

      1350        1360        1370        1380        1390
       *           *           *           *           *
GCT TTT ACT GTG TGG ATT ATG CCA ACA GCT TGA ATG GAT TCT AAC AGA

      1400        1410        1420        1430        1440
       *           *           *           *           *
CTC TTT TGT TTT TAA AAG TCT GCC TTG TTT ATG GTG GAA AAT TAC TGA

           1450        1460        1470        1480
            *           *           *           *
AAC TAT TTT ACT GTG AAA CAG TGT GAA CTA TTA TAA TGC AAA TAC TTT

1490        1500        1510        1520        1530
 *           *           *           *           *
TTA ACT TAG AGG CAA TGG AAA AAT AAA AGT TGA CTG TAC TAA AAA TGT

 1540        1550        1560        1570        1580
  *           *           *           *           *
ATA CTT GTT GCC AGG AAG GTG ACC TCA AAA ATT AAA AGT ATA ATT ATT

      1590        1600        1610        1620        1630
       *           *           *           *           *
CGG CCG GGC ATG GTG GCT CAC ACC TGT AAT TCC AGC ACT TTG GGA GGC

      1640        1650        1660        1670        1680
       *           *           *           *           *
CAA GGC AGG CGG ATC ACG AGG TCA GGA GTT CAA AAC CAG CCT GTC CAA

TAT AGT G
```

FIG.3 (cont'd.)

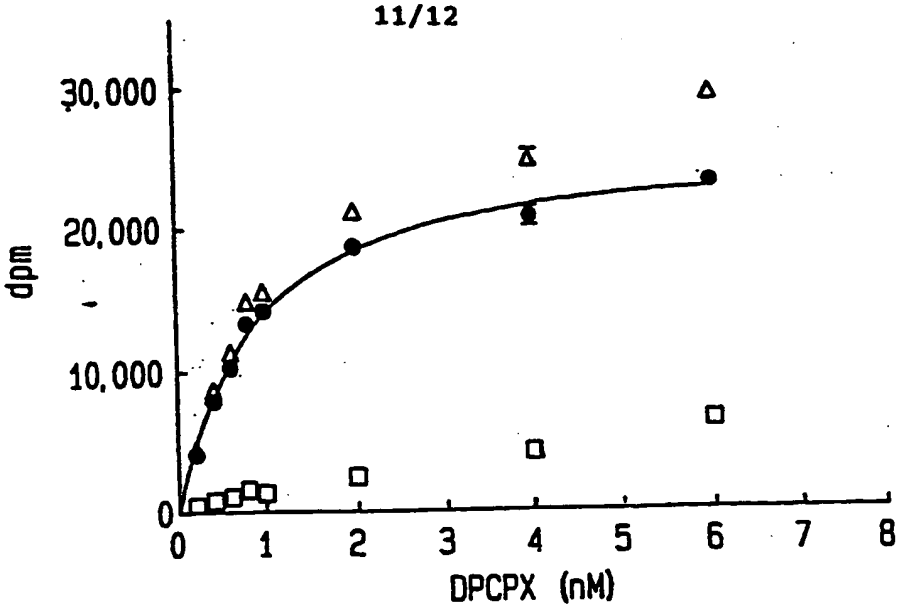
30,000
20,000
10,000
0
dpm
0 1 2 3 4 5 6 7 8
DPCPX (nM)

FIG.4a

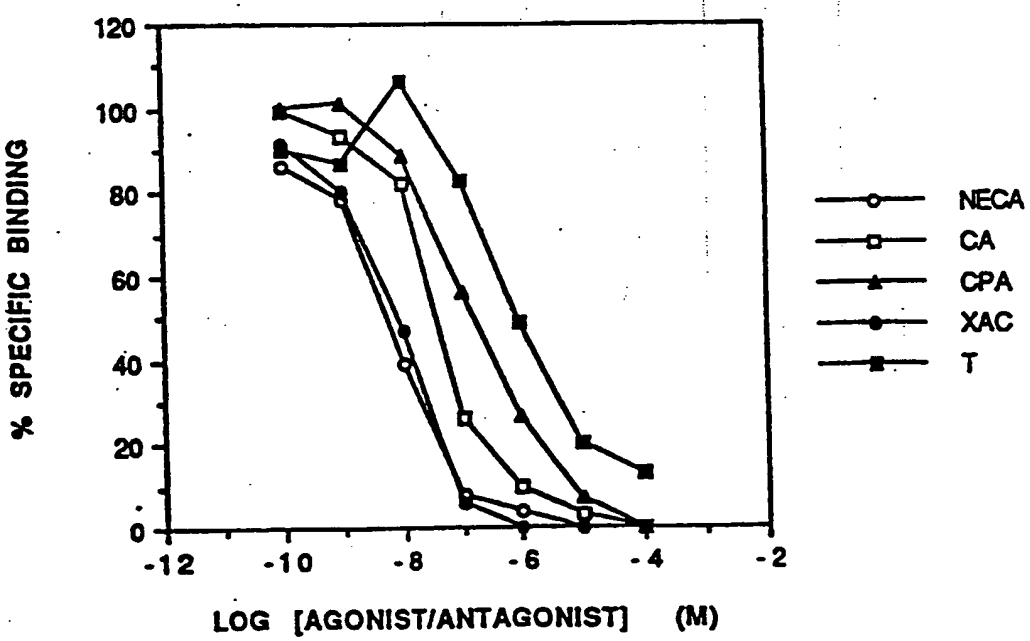
120
100
80
60
40
20
0
% SPECIFIC BINDING
-12 -10 -8 -6 -4 -2
LOG [AGONIST/ANTAGONIST] (M)
NECA
CA
CPA
XAC
T

FIG.4b

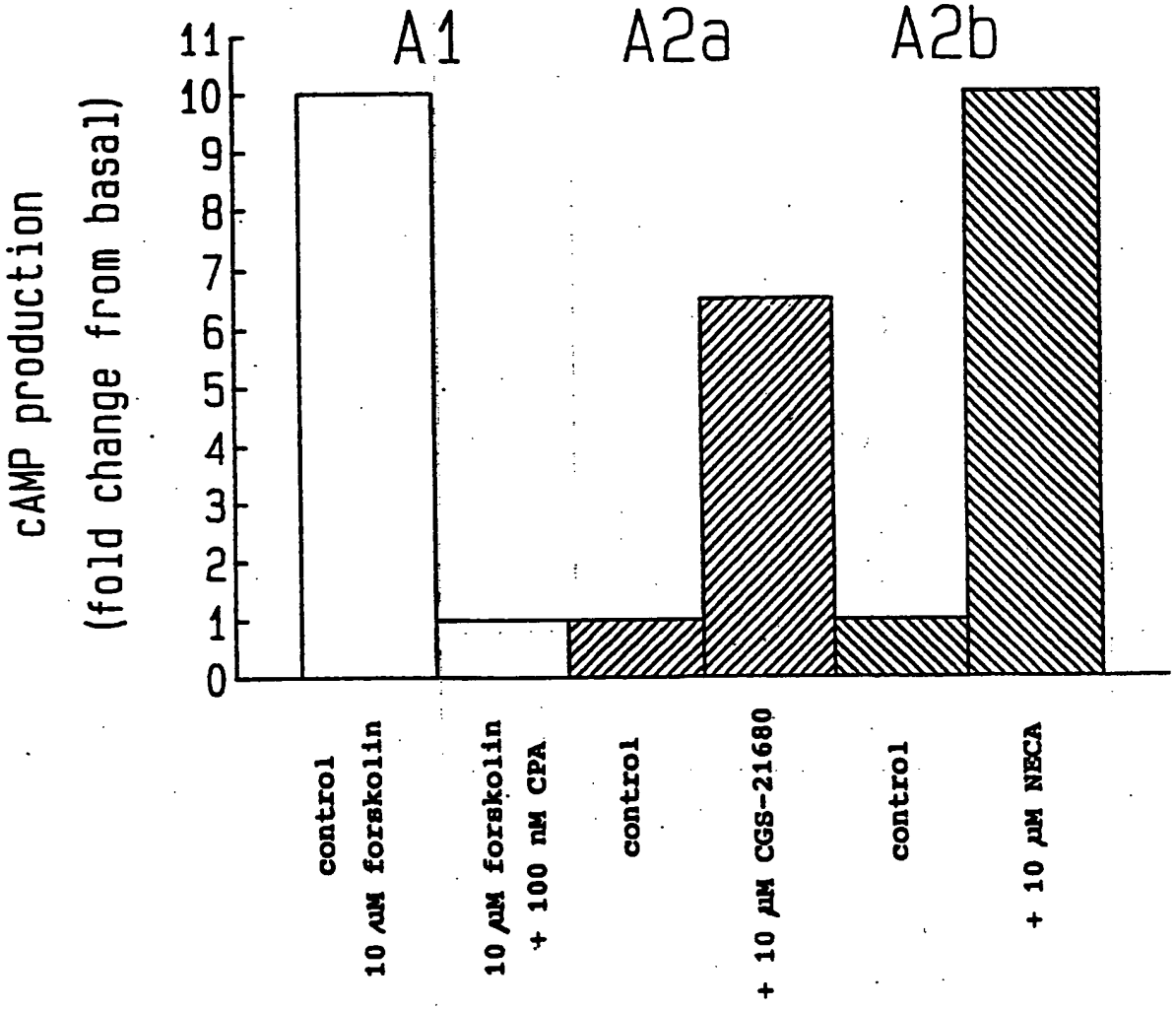
A1
A2a
A2b
cAMP production
(fold change from basal)
0
1
2
3
4
5
6
7
8
9
10
11
control
10 μM forskolin
10 μM forskolin
+ 100 nM CPA
control
+ 10 μM CGS-21680
control
+ 10 μM NECA

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 93/00277

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl.[5] C12N 15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[5]: C12N 15/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC C12N 15/12

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
Derwent Database: WPAT - Keywords Adenosin: ADE, Receptor, C12N
BIOT - Keywords Adenosin: ADE, Receptor
CASA - Keywords Adenosin: ADE, Receptor, DNA or Gene, A1, A2A or A2B

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	AU,A,21791/92 (THE UNITED STATES OF AMERICA REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 10 December 1992 (10.12.92)	1-7
Y	AU,A,75792/91 (THE UNITED STATES OF AMERICA REPRESENTED BY THE SECRETARY, U.S. DEPARTMENT OF COMMERCE) 31 October 1991 (31.10.91)	1-7
Y	GENOMICS 11,225-227 (1991) CHROMOSOMAL MAPPING OF A1 & A2 ADENOSINE RECEPTORS, VIP RECEPTOR, & A NEW SUBTYPE OF SEROTONIN RECEPTOR, Published 1991	1-7

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 1993 (26.08.93)	2 SEP 1993 (2.09.93)
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (06) 2853929	Authorized officer JOHN ASHMAN Telephone No. (06) 2832364

Form PCT/ISA/210 (continuation of first sheet (2)) (July 1992) copljm

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
A	AU,A,52215/90 (MERRELL DOW PHARMACEUTICALS INC) 4 October 1990 (04.10.90)	

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	92/21701	AU	21791/92
WO	91/16056	AU	75792/91

END OF ANNEX